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Solidification mechanisms of chitosan-glycerol phosphate/blood implants for articular cartilage repair

C. Marchand[1], G. Rivard[2], J. Sun[3], C.D. Hoemann[4];
[1]Institute Of Biomedical Engineering, Ecole Polytechnique, Montreal, Canada, [2]Division Of Hematology-oncology, Hôpital Sainte Justine, Montreal, Canada, [3]Cartilage Repair, Bio Syntech Canada Inc, Laval, Canada, [4]Dept Chemical Engineering, Ecole Polytechnique, Montreal, Canada

Purpose: Chitosan-glycerol phosphate (chitosan-GP, or BST-CarGel™) is a unique chitosan polymer solution that is mixed with whole blood and solidified over microfracture defects to stimulate cartilage repair. The purpose of this study was to elucidate the mechanisms underlying chitosan-GP/blood implant solidification.
Methods and Materials: Chitosan-GP/blood mixtures and unmodified whole human blood, with or without added clotting factors, were evaluated with a Thromboelastograph® for clotting time and clot tensile strength. Serum was collected from solidifying clots and analyzed for platelet factor 4, activated thrombin (thrombin-antithrombin), and FXIIIa. Solidification of chitosan-GP/blood mixtures, with and without clotting factors, was observed in vivo in microdrilled cartilage defects of skeletally mature rabbits (n=4 to 6 per condition).
Results: Chitosan-GP/blood mixtures experienced an atypical biphasic increase in clot tensile strength. Within the first 10 minutes, chitosan-GP/blood mixtures acquired low tensile strength, and contained partly activated platelets and agglutinated red blood cells in the absence of detectable thrombin-antithrombin generation. An increase in clot tensile strength at 20 to 40 minutes was concomitant with thrombin activation, a burst of platelet activation, and FXIIIa generation. Addition of tissue plasminogen activator depressed whole blood and chitosan-GP/blood clot tensile strength, demonstrating a role for fibrin formation in implant solidification. Addition of activated clotting factors accelerated chitosan-GP/blood solidification in vivo (2.2 vs 4.2 minutes, $p<0.05$).
Conclusions: Chitosan-GP/blood implants solidify through mechanisms involving thrombin activation and natural coagulation processes including platelet activation and fibrin fiber formation. Clotting factors can be used to shorten implant in situ solidification time in microdrilled articular defects.

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The intra-articular location of a partial thickness cartilage defect influences the natural history of cartilage filling of the defect.

S. Heir[1], A. Årøen[2], S. Loeken[3], F.P. Reinholt[4], L. Engebretsen[2];
[1]Norges Idrettshogskole, Oslo Sport Trauma Research Center, Oslo, Norway, [2]Orthopaedic Centre, Ullevål University Hospital, Oslo, Norway, [3]Orthopaedic, Ullevaal university hospital, Oslo, Norway, [4]Pathology, Rikshospitalet, Oslo, Norway

Purpose: To investigate the difference in natural history cartilage filling related to the intra-articular location of an experimental induced cartilage defect in the rabbit knee.
Methods and Materials: A partial thickness cartilage defect (¢ = 4 mm) was induced in both knees of 35 adult New Zealand rabbits at the age of 22 weeks. The animals were randomized to having the defect induced in the patella in either the right or left knee, whereas the other knee had the defect induced in the medial-femur-condyle (MFC). An operating microscope was used to secure the preparation of the defects. The animals were sacrificed at 12 weeks (n=8), 24 weeks (n=9), and 36 weeks (n=18) after surgery. Analysis® software was used in measuring the cartilage height at 7 determined spots in each defect. Cartilage filling was estimated relating the mean cartilage height in each defect to the mean cartilage height of the shoulders of the same defect. Statistical analyses were performed using Student T-test.
Results: Twelve weeks after surgery the cartilage filling of patella defects were 32.3%, compared to 16.9% in MFC defects ($p<0.05$). Twenty-four weeks after surgery the filling of patella defects were 25.0%, compared to 8.0% in MFC defects ($p<0.05$). Thirty-six weeks after surgery the filling of patella defects were 48.8%, compared to 20.5% in MFC defects ($p<0.05$). There was a significant rise in cartilage filling from 12 to 36 weeks post surgery at both locations.
Conclusions: The intra-articular location of a partial thickness cartilage defects influences the natural history of cartilage filling of the defect.

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Human auricular cartilage – an alternative source of chondrocytes for autologous chondrocyte implantation (ACI)

E. Maličev[1], **A. Barlič**[2], N. Kregar-Velikonja[1], A. Alibegović[3], M. Drobnič[4];
[1]Educell D.o.o., Educell d.o.o., Ljubljana, Slovenia, [2]Educell d.o.o., Ljubljana, Slovenia, [3]Institute Of Forensic Medicine, Medical Faculty, Ljubljana, Slovenia, [4]Dept Of Orthopaedic Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: Autologous chondrocyte implantation (ACI) is a promising tissue-engineering method for reconstruction of articular cartilage lesions. One disadvantage of the method is that a biopsy has to be taken from a healthy, less weight bearing area of the cartilage, causing morbidity at the donor site. An alternative source of chondrocyte would thus be an advantage.
Methods and Materials: Cell cultivation: Human articular and auricular cartilage was obtained from donors post-mortem (approved by National Medical Ethics Committee; code 74/12/01). Chondrocytes were isolated by collagenase II digestion and seeded into monolayer cell culture and 3-dimensional alginate hydrogel system. Cells were grown in DMEM medium supplemented with 15% human serum for 1 week. Quantification of gene expression: Total RNA from the cell was isolated by TRI REAGENT™ (Sigma, USA) and cDNA was prepared using High Capacity cDNA Archive Kit (Applied Biosystems, USA). All primers and probes for collagen type I, collagen type II, aggrecan, versican, elastin, GAPDH for quantitative real-time PCR were from Applied Biosystems, USA.
Results: Results based on four experiments showed, that differences in mRNA expression of analyzed genes between articular and auricular chondrocytes are higher during monolayer culturing. When the isolated cells were seeded in alginate hydrogel without previous monolayer expansion, their phenotype profile was more comparable. As expected, synthesis of collagen type II and aggrecan in alginate hydrogel was upregulated in comparison to monolayer culture in both cell types.
Conclusions: According to preliminary results, auricular chondrocytes might be used as an alternative source of the cells for articular repair.

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Use of autologous costal chondrocytes transplantation in the repair of defects of the rabbit articular cartilage

P. Szeparowicz[1], J. Popko[2], S. WoŁczyński[3], B. Sawicki[4];
[1]Department Of Children Orthopedy, Medical Academy BiaŁystok, BiaŁystok, PODLASKIE, Poland, [2]Children Orthopedy, Medical Academy BiaŁystok, BiaŁystok, Poland, [3]Ginecological Endocrynology, Medical Academy BiaŁystok, BiaŁystok, Poland, [4]Histology Department, Medical Academy BiaŁystok, BiaŁystok, Poland

Purpose: The treatment of articular cartilage defects remains an unsolved problem of today's medicine. In our experiment we tried to assess costal chondrocytes' potential to induce repair of full-thickness defects in the articular cartilage of a rabbit femur
Methods and Materials: An artificially made, full-thickness defect with a preserved subchondral bone in the rabbit's femoral patellar groove was created. Autologous cultured costal chondrocytes were then transplanted into the defects and covered with periosteal flaps. Empty defects and defects with periosteum alone were created in a control groups. Three groups have been formed (ten rabbits each), which were examined at four, twelve and twenty-four weeks after transplantation, respectively. The reparative tissue was evaluated by macroscopic, histological, and immunohistochemical examination and were graded with scale described by Wakitani et al.
Results: The reparative tissues in defects with transplanted chondrocytes had mostly a hyaline-like cartilage appearance and were firmly attached to surrounding normal cartilage. A positive immunohistochemical reaction with collagen type II antibody was observed throughout the repair tissue. No trace of newly formed bone was detected. The reparative tissues in control defects, had a fibrous character, were loosely connected to the surrounding cartilage, and showed negative immunohistochemical reaction with collagen type II antibody. Statistically significant correlation between main group and controls groups were found.
Conclusions: Considering these findings, the ease of surgical procedures during the harvesting of the costal cartilage and the lower number of interventions into the joint, we have found costal chondrocytes a promising material for transplantation. However we understand that this method needs further examination.